Finding Solutions for the Challenges Facing T-Cell Therapies for Malignancy

CAGT
CENTER FOR CELL AND GENE THERAPY

Baylor College of Medicine

Texas Children's Hospital

Houston Methodist
LEADING MEDICINE

Foundations of Cancer Therapeutics (FCT)

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Disclosures

- SFI
  - Founder member AlloVir and Marker Therapeutics
  - Bellicum Pharmaceuticals
- Spouse
  - Allogene, Allovir, Abintus, Bluebird Bio Inc, Marker Therapeutics, Memgen LLC, TScan, Turnstone Biologics Ltd and Walking Fish

Successes of T-cell Immunotherapies

- Robust and durable cures
  - Tumor Infiltrating lymphocytes (TILs)
    - Melanoma, ovarian, cervical non small lung….
  - Virus-specific T-cells after hematopoietic stem cell transplantation
    - CMV, EBV, adenoviruses, BK, HHV6, COVID19
  - CD19 CAR T-cells for leukemia and lymphoma
  - BCMA.CAR T-cells for multiple myeloma

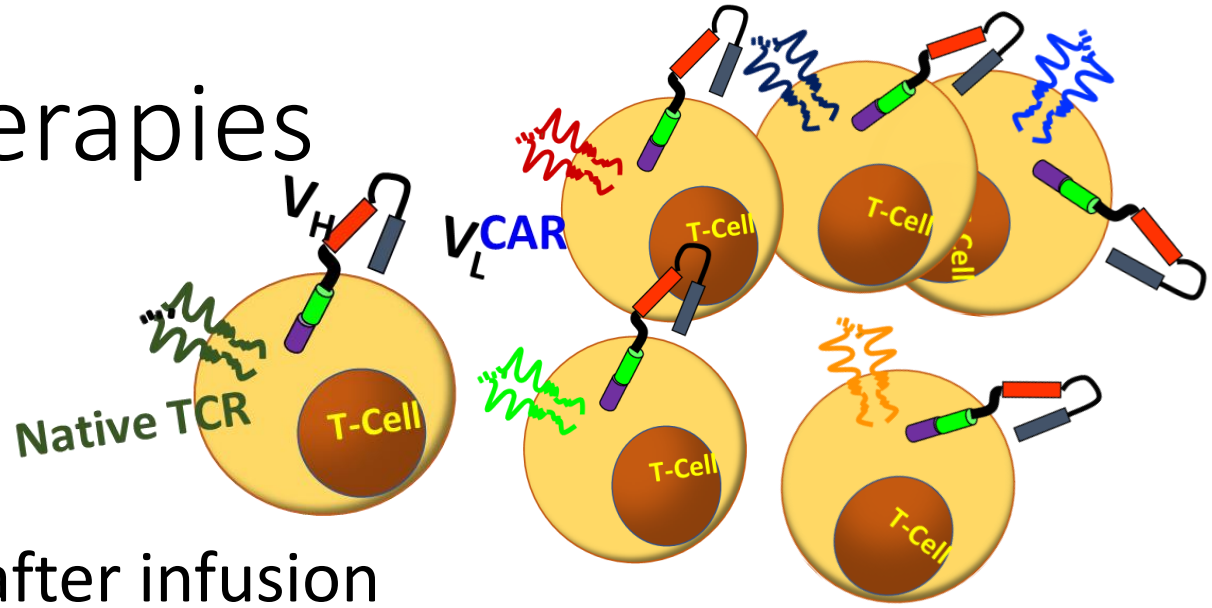
Why bother with T-cell Immunotherapies?

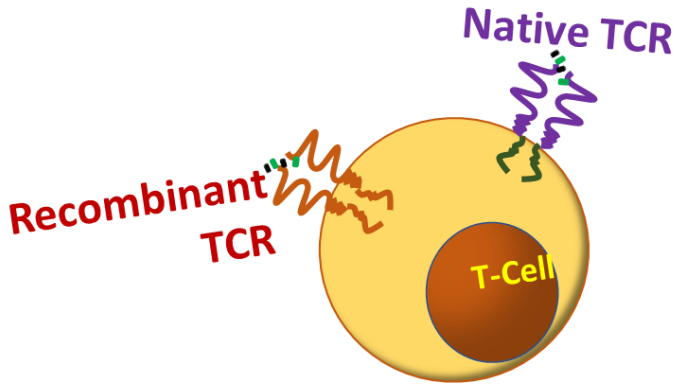
- Requires GMP manufacturing
  - Expensive, cumbersome, time consuming
- Exquisite specificity of T-cell killing
- Lack the severe long-term toxicities of standard chemoradiotherapies
  - Hair loss, infertility, cardiomyopathies, skeletal abnormalities, second malignancies
- Avoid often debilitating surgeries

- CD19.CARTs now 2nd line therapy

Challenges to T-cell Therapies

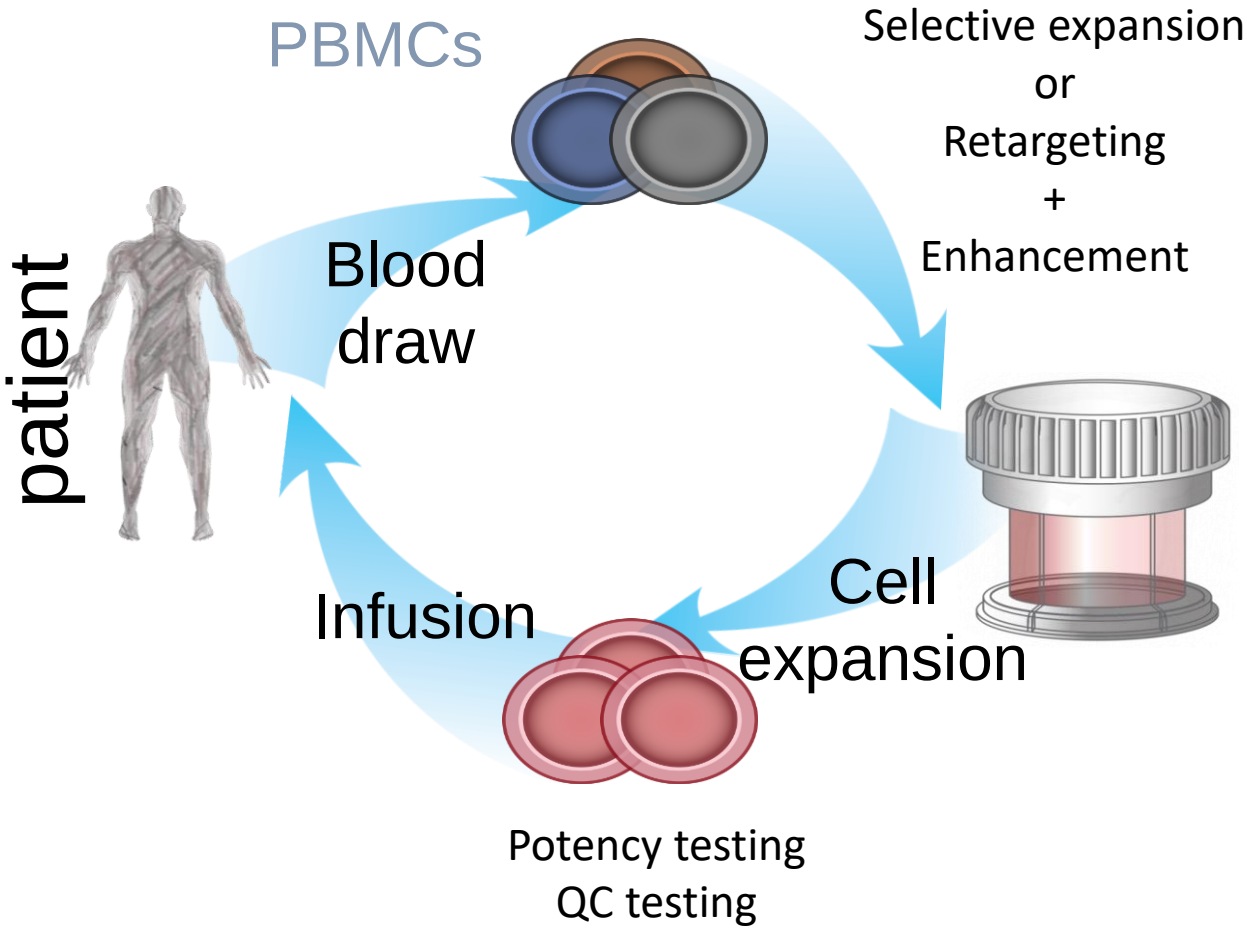
- Monospecifity of CAR T-cells
  - Leading to antigen loss
  - Bi/Tri-specific CARs
- Lack of appropriate stimulation after infusion
  - T-cell exhaustion/dysfunction/contraction
  - Genetic modifications
  - Combination with small molecules
- Immunosuppressive tumor microenvironment
  - Inhibitory cytokines, ligands and cells
  - T-cell exhaustion dysfunction
  - Genetic modifications





Challenges to T-cell Therapies

- Patient derived products
  - T-cells damaged by multiple chemoradiotherapies
  - Manufacturing time
    - Up to 6 weeks for procurement, expansion and release testing
    - Too long for patients with acute need
  - Expense of single patient products

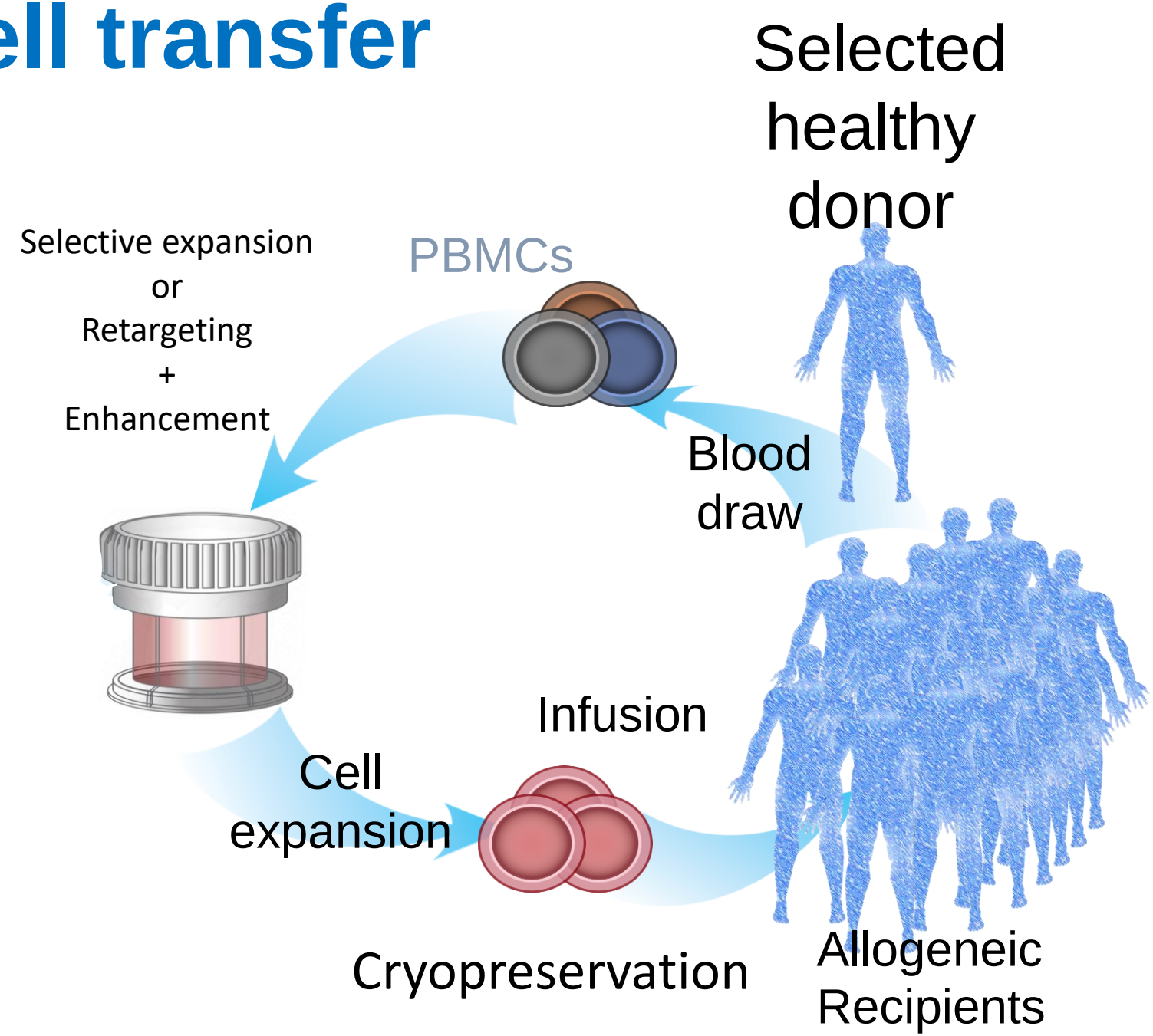


Banked therapeutic T-cells

- Rationale
- Challenges
- Solutions
- Clinical implementation
  - Problems
  - Potential solutions

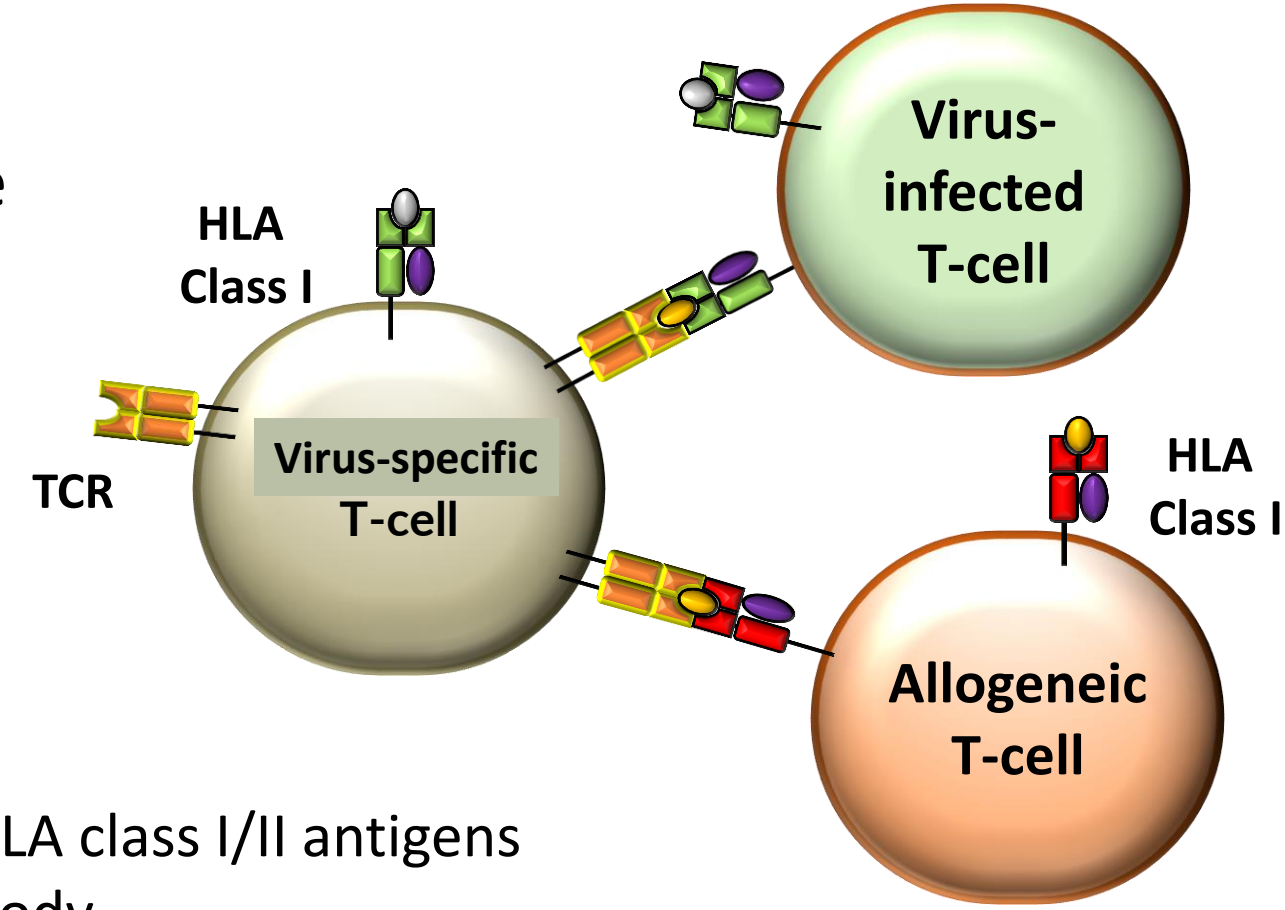
Adoptive T-cell transfer

- Healthy donors selected for T-cell potency
- PBMCs show better expansion and function
- One donor can potentially produce sufficient T-cells for 100’s of patients
- Immediately available



Challenges of Allogeneic T-cell therapies

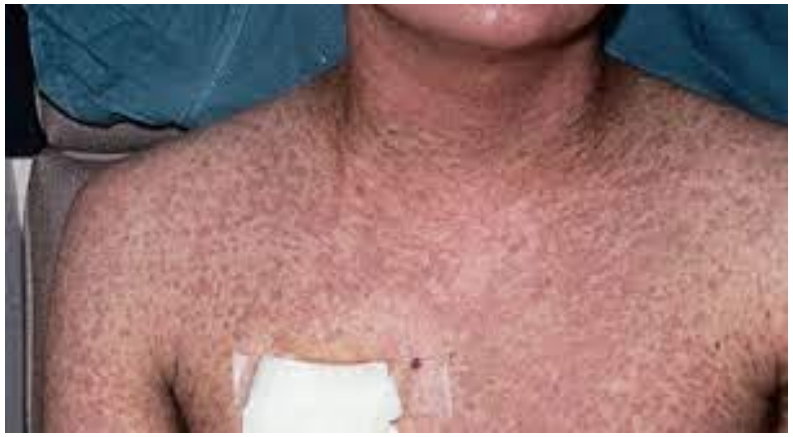
- Alloreactive T-cells/NK cells
- TCR recognizes self HLA:peptide combination
- High affinity self-specific TCRs deleted in thymus
- Allospecific TCRs not deleted
  - Emerge as naïve T-cells



- 10% of T-cells recognize allogeneic HLA class I/II antigens
  - ~4 x 10^{11} T-cells in the human body
  - ~4 x 10^{10} may be alloreactive

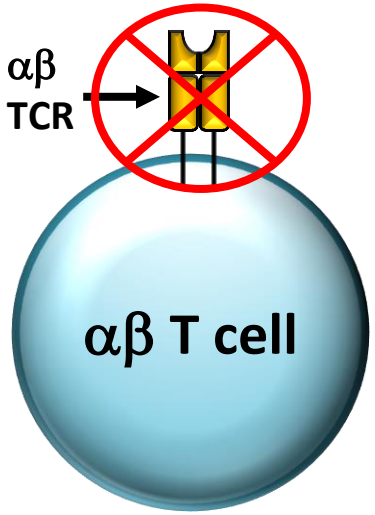
Alloreactive T-cells cause graft versus host disease (GVHD) and graft rejection

- Alloreactive T-cells in donor product
  - Cause GVHD
- Recipient alloreactive T-cells
  - Cause graft rejection
- Majority of alloreactive T-cells reside in the naïve T-cell compartment

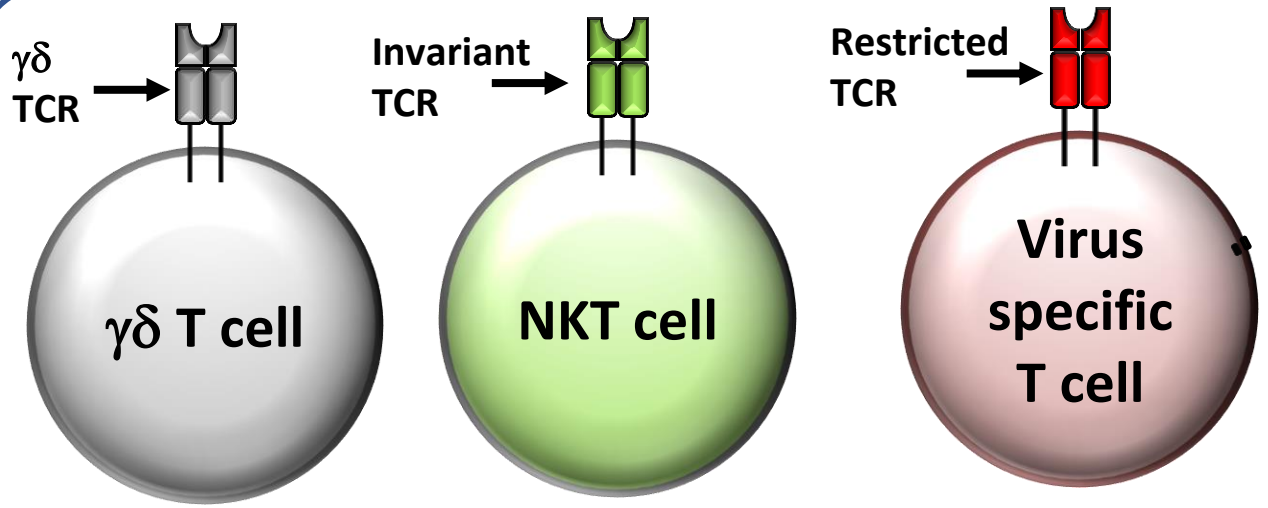


GVHD post HSCT

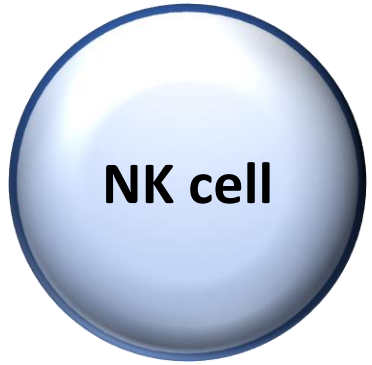
Off-the-Shelf Platforms That Lack GVHD



- Highly polyclonal TCRs (~10^8)
- Most common T-cell therapies
- Knockout TCR to eliminate alloreactivity



- Can use T-cells with restricted or invariant TCRs



- Can also use Natural Killer (NK) cells that lack TCR

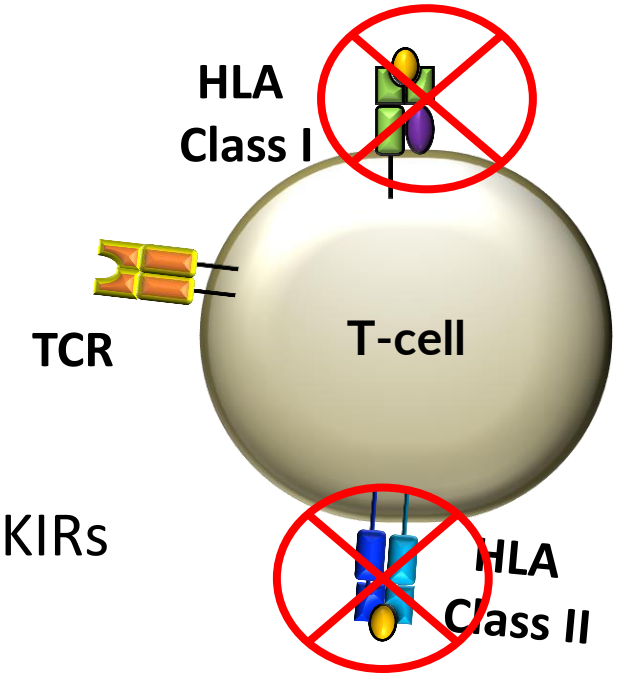
Allogeneic virus-specific T-cells (VSTs) have been safe and effective

- In the HSCT setting
  - Partially HLA matched
    - Recognize viral antigens through shared HLA antigens
  - Up to 90% efficacy against multiple viruses
  - No GVHD
- EBV-specific T-cells (EBVSTs) for EBV+ lymphoma
  - Partially HLA-matched
  - Patients not immunosuppressed
  - High response rates including 4 CRs in 14 patients
  - No GVHD
- VSTs derive from memory compartment
  - Lower TCR diversity then naïve compartment
  - Lower chance of GVHD than from CD3/28-activated T-cells

V_H V_L

Common Strategies to Prevent Graft Rejection

- Knockout major histocompatibility antigens
  - HLA A, B, C (class I), DR, DP and DQ (class II)
- HLA class I -ve cells susceptible to NK cells
  - Express HLA E-β2M chimera
    - Heterogeneous expression of inhibitory and activating KIRs
    - Insufficient to protect against all NK cell subsets



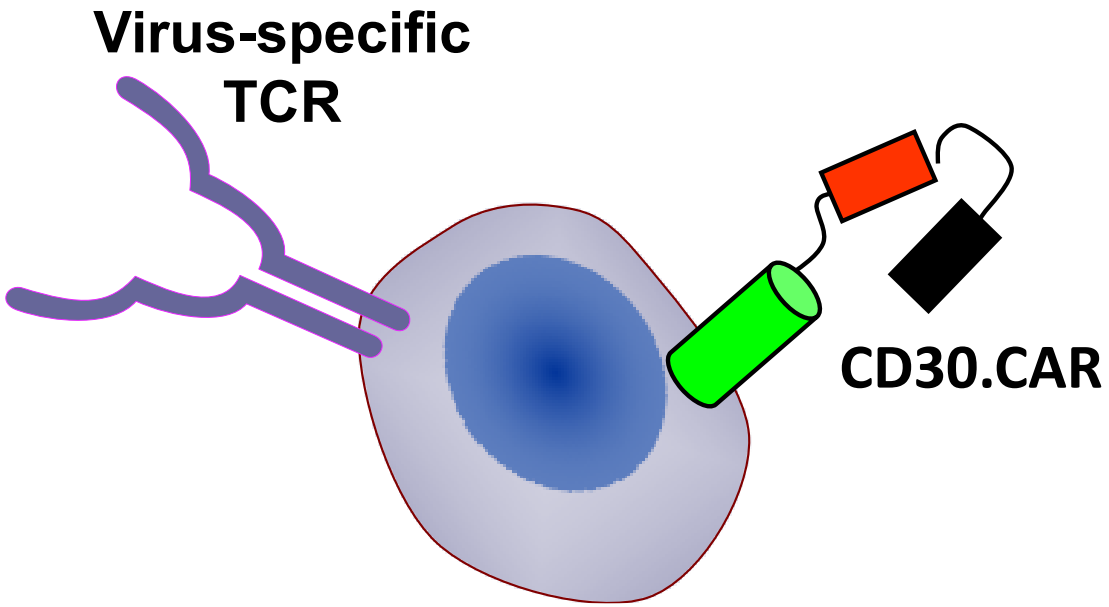
Problems with Gene Editing

- Most strategies involve gene editing
  - Chromosomal rearrangements, mutations
  - Concern for oncogenicity
  - Regulatory issues

Our Strategy

- Epstein-Barr virus specific T-cells (EBVSTs)
  - To avoid GVHD
- CD30.CARs to prevent rejection
- Avoids gene editing

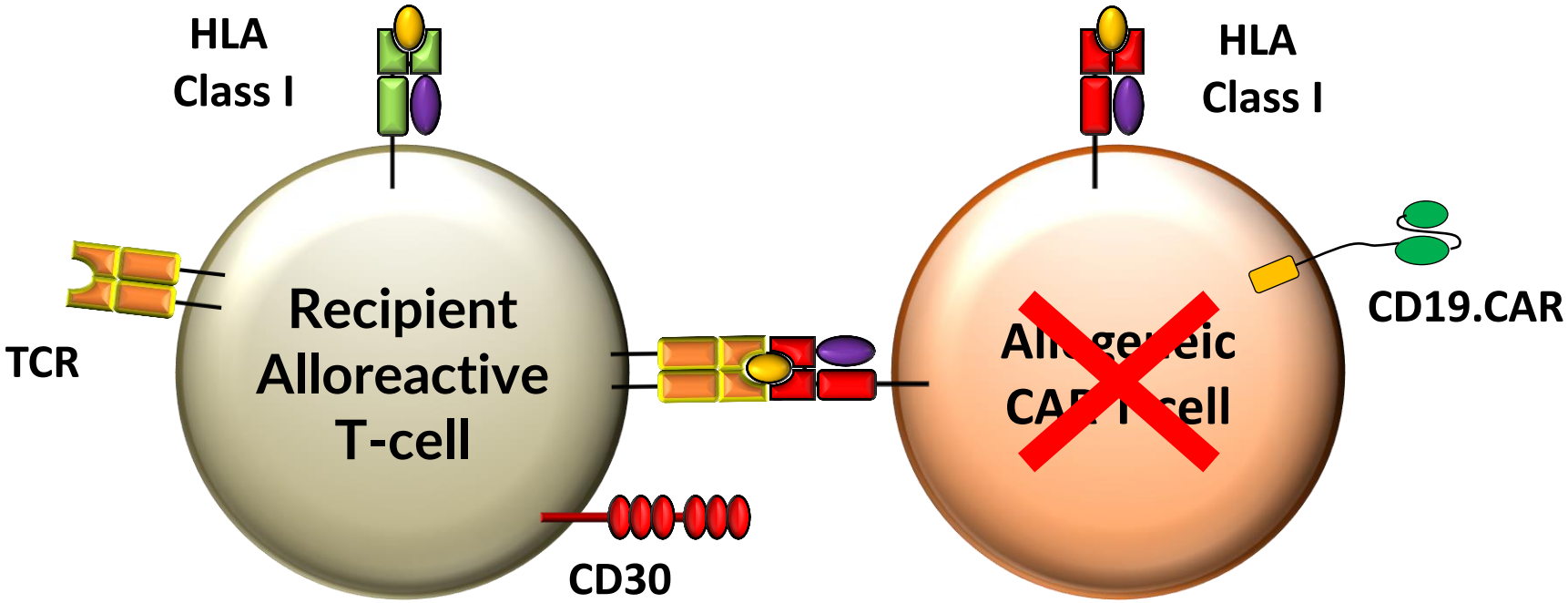
David Quach



CD30.CAR to Prevent Graft Rejection

- CD30 expression induced on activated T cells
  - Including allo-activated T cells
  - CD30.CAR-T cells will kill alloreactive T cells they encounter
- CD30.CAR-Ts have been evaluated clinically
  - Ramos et al J Clin Oncol. 2020 Nov 10;38(32):3794-3804.
  - Effective against CD30+ lymphoma
    - OR 26/36 (72%), CR 20/36 (55%)
  - Safe
    - Minimal CRS
    - No increase in viral infections or reactivations

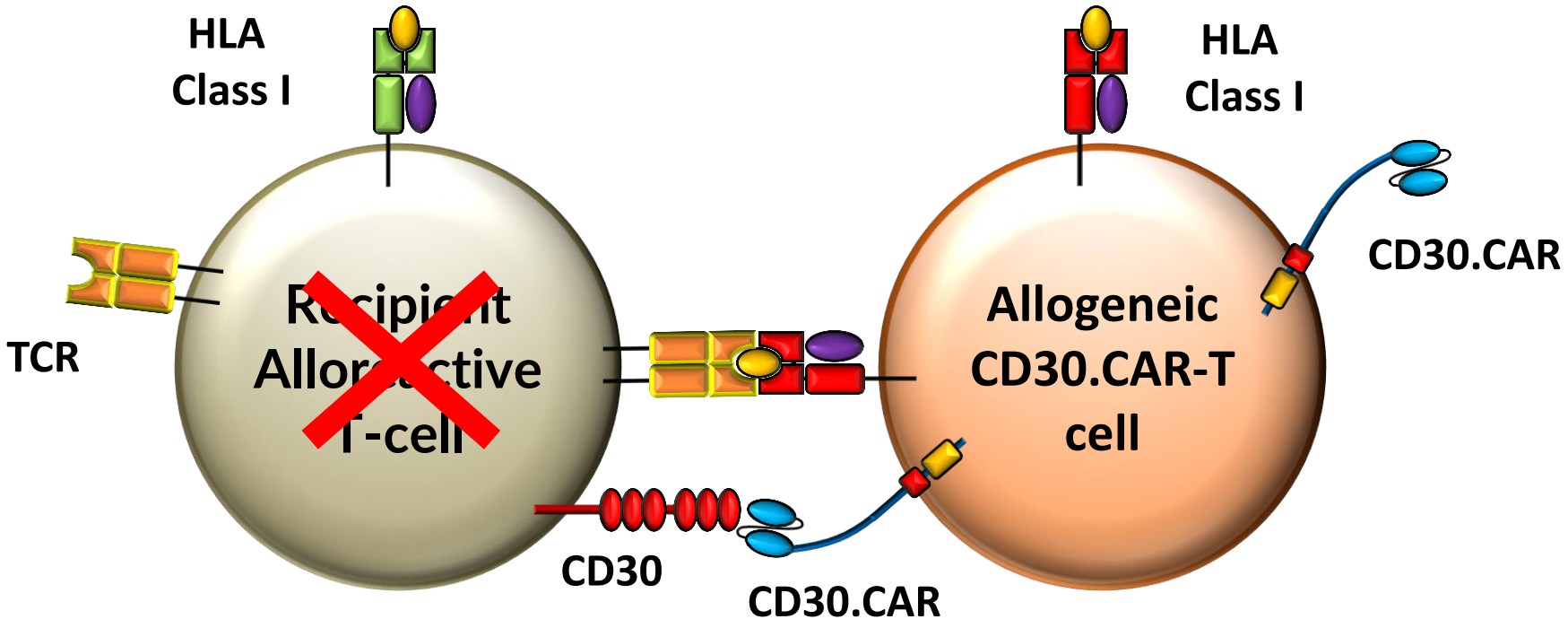
Recipient Alloreactive T Cell Rejects donor Allogeneic T Cell



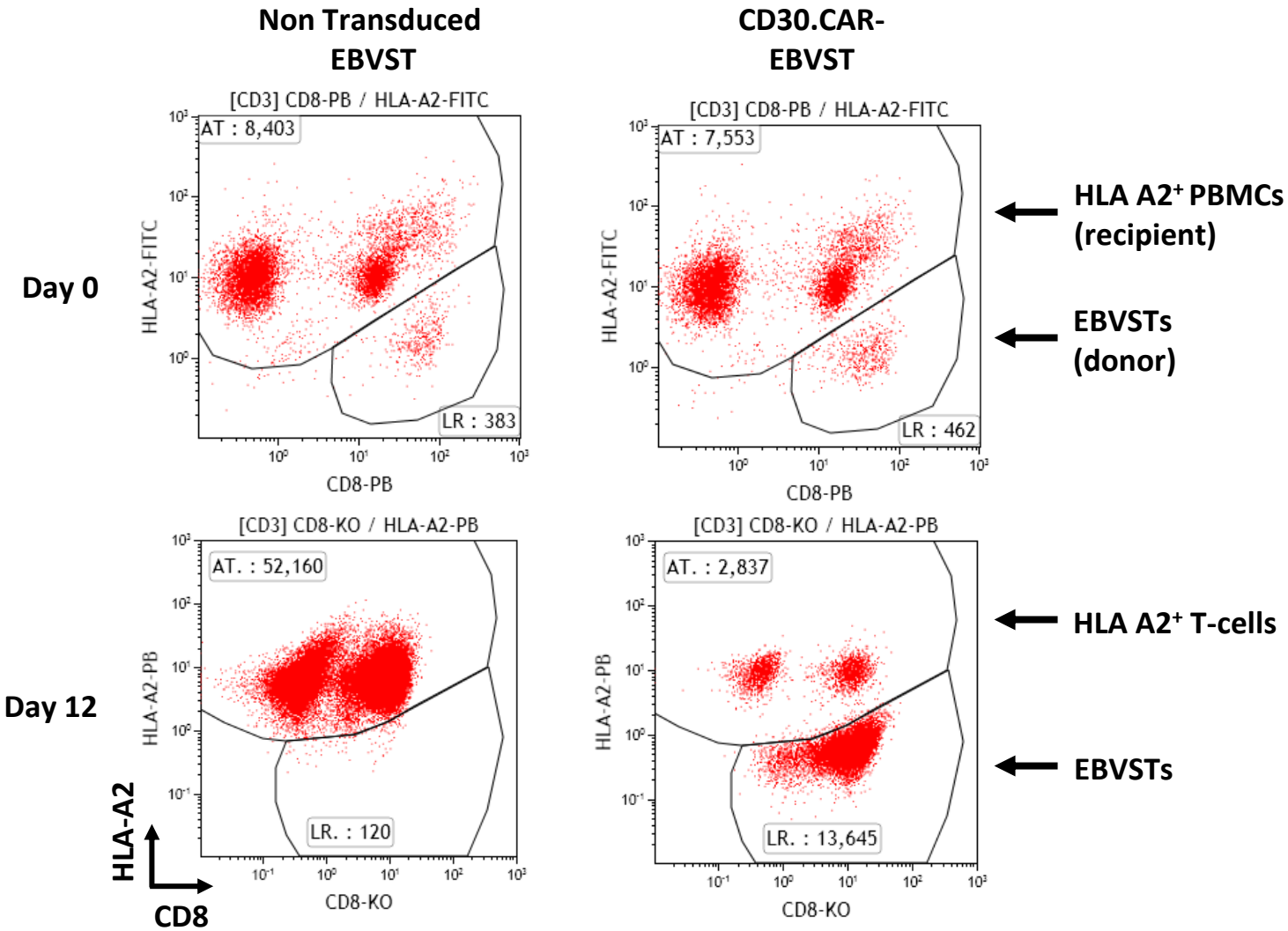
Alloreactive T-cells recognize allogeneic HLA

- Become activated and express activation markers
  - Like CD30
- Expand and kill allogeneic cells

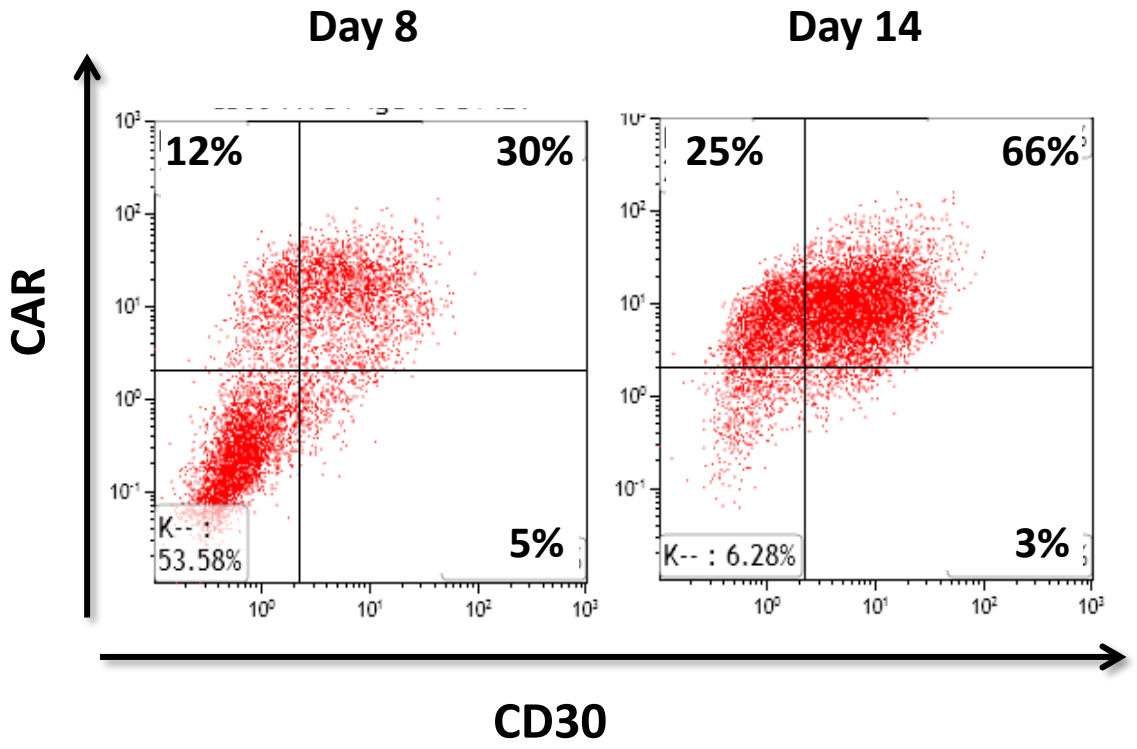
Allogeneic CD30.CAR-T Cell Kills Host Alloreactive T-Cell

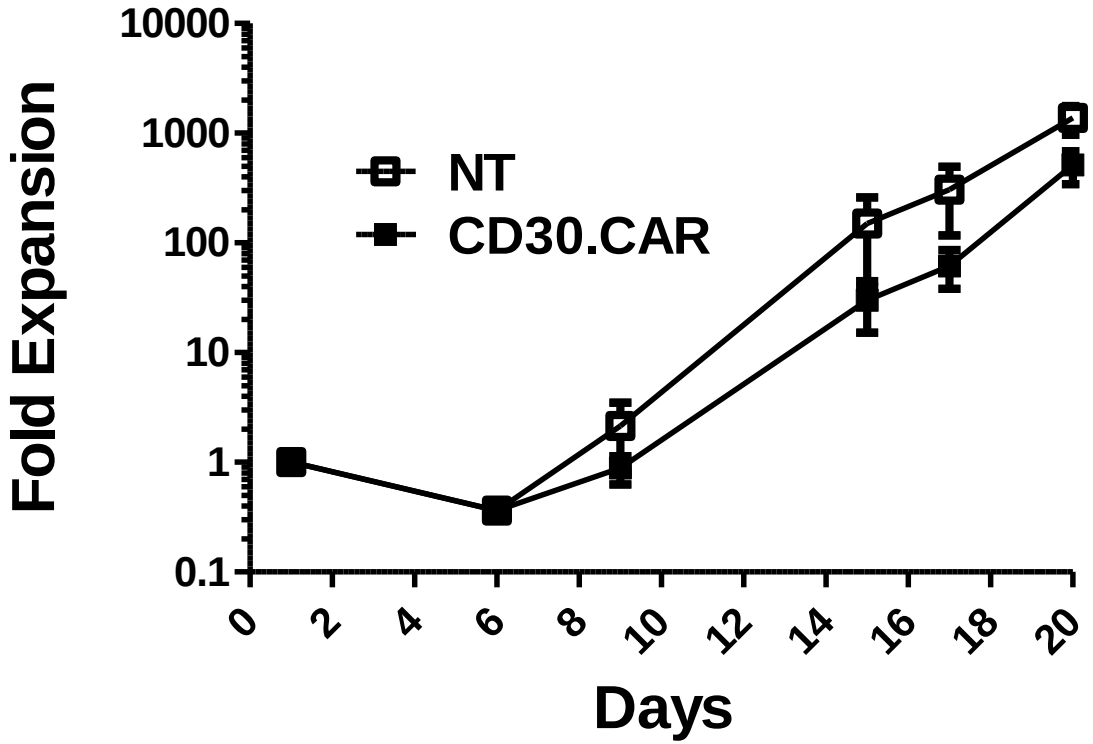


CD30.CAR Protects EBVST from Allo PBMCs in Co-Cultures



CD30 Also Expressed in CD30.CAR-EBVSTs!!

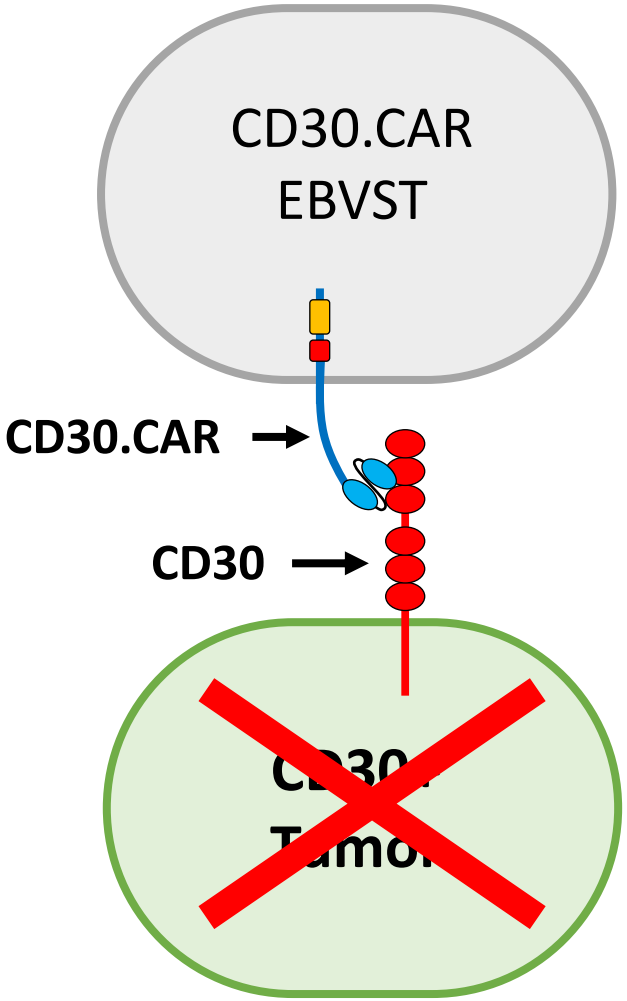




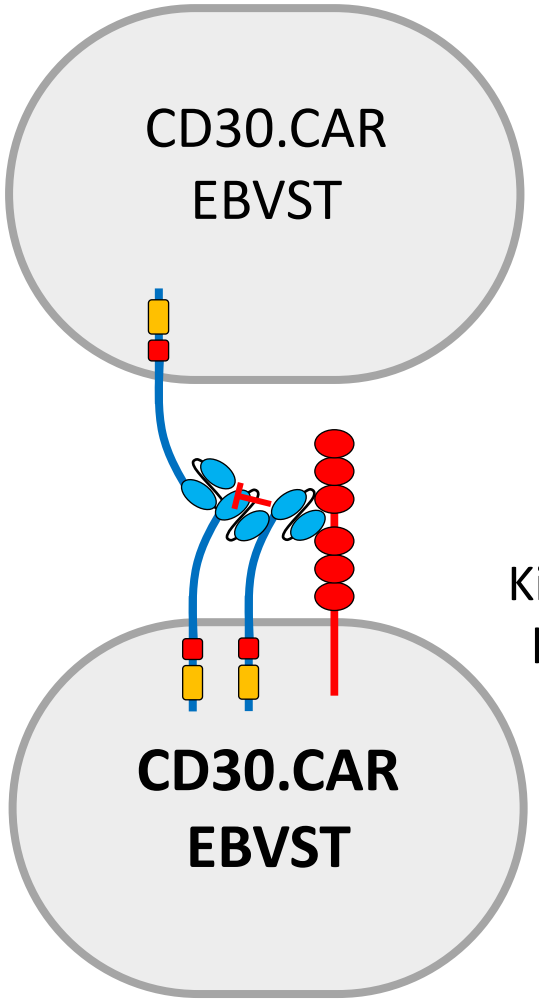
How can EBVSTs express CD30 and survive?

CD30.CAR masks CD30 in CIS

CD30.CAR EBVSTs
targeting CD30+ tumors



Masking prevents killing *in trans*

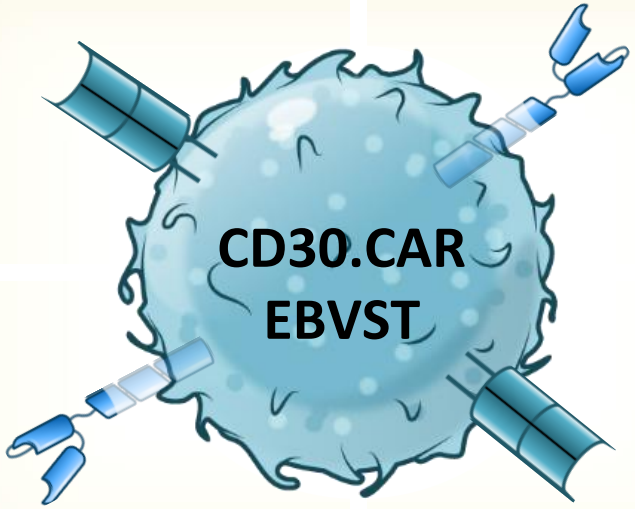


Killing does not
happen *in cis*

Rationale for CD30.CAR-EBVSTs

VSTs are safe[1]
- No GVHD

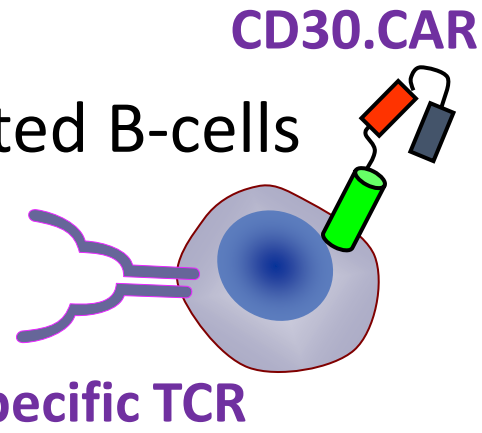
Kill alloreactive T-cells[2]
- No Rejection



Kill CD30+ tumors[3]
- EBV+
- EBV-

Kill EBV+ tumors[3]
- CD30+
- CD30-

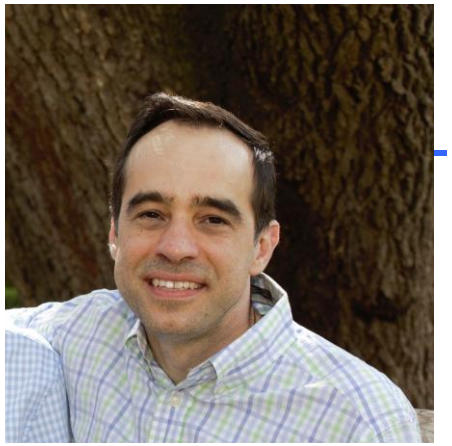
Partial HLA matching of donor EBVSTs with recipient



- Stimulation of CD30.CAR-EBVSTs by recipient EBV-infected B-cells
  - via the EBV-specific TCR
- Additional targeting of tumors if EBV+
- Partial HLA matching does not prevent rejection
  - Would need complete HLA identity with donor
    - Would need a massive bank
- Not necessary for targeting CD30

Phase I trial to evaluate OTS CD30.CAR EBVSTs

- Safety
- Anti-tumor activity
- Persistence of CD30.CAR EBVSTs
- 4 weeks between patients
- Only one dose of T-cells



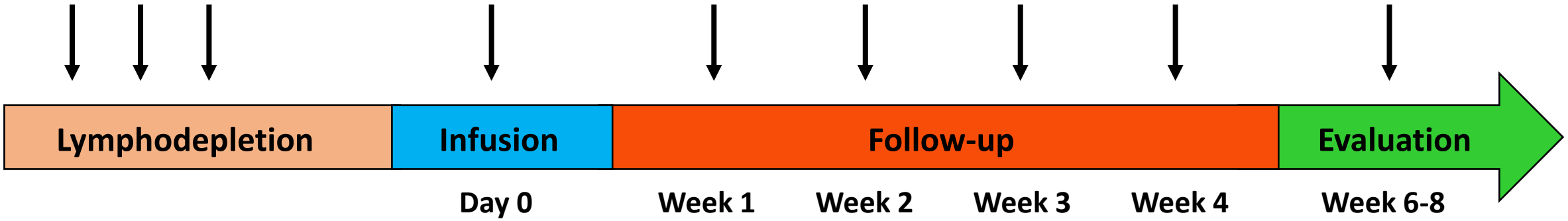
Carlos Ramos

Cyclophosphamide
-500mg/m2/day
Fludarabine
-30mg/m2/day

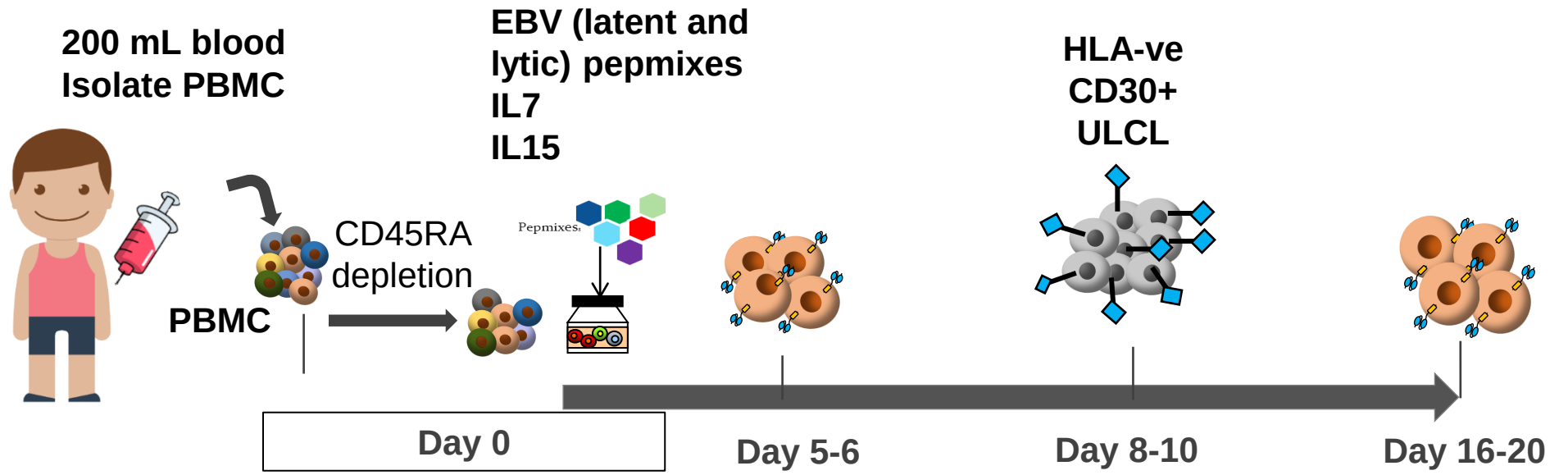
Three dose levels
-DL1: 4 x 10^7 cells
-DL2: 1 x 10^8 cells
-DL3: 4 x 10^8 cells

Draw blood weekly
-Measure transgene
-Evaluate epitope spreading

Diagnostic Scans
-PET/CT



Banked EBV-Specific T-Cells Expressing CD30.CAR for Allogeneic Recipients (BESTA)



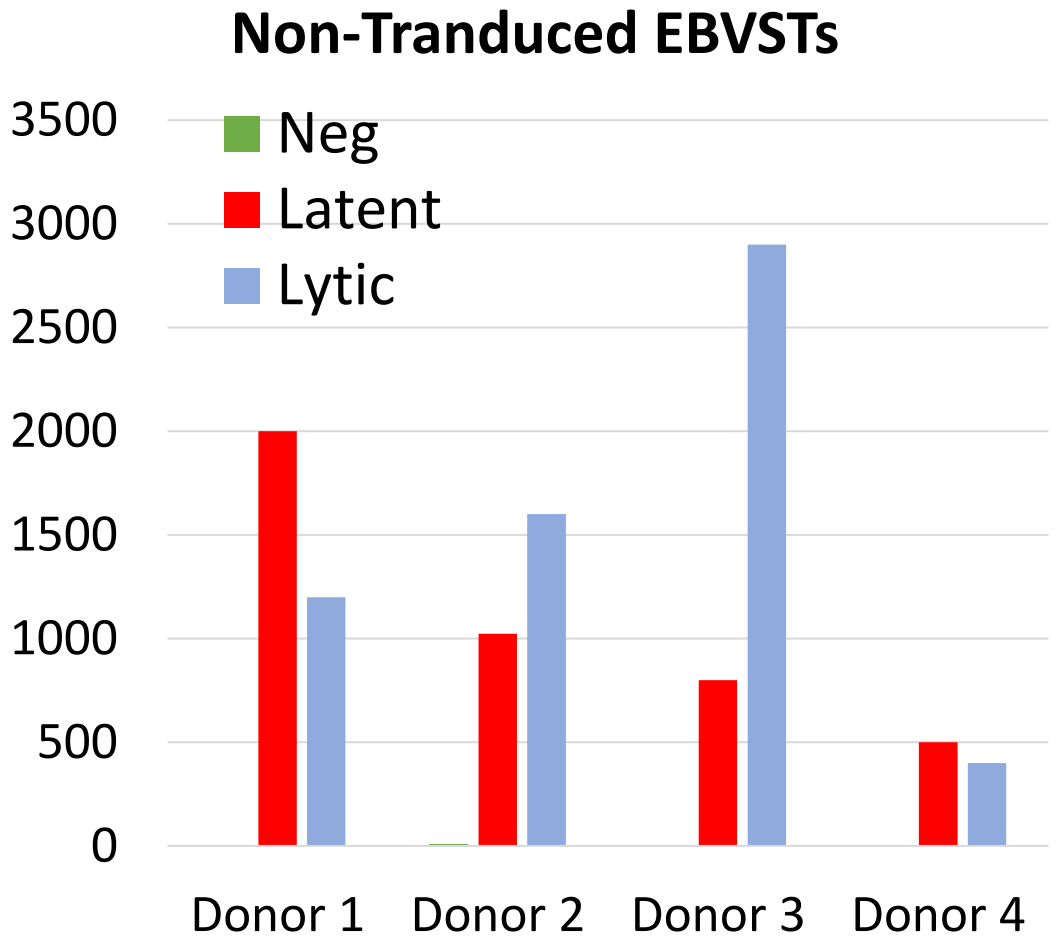
Characterization: 7 lines generated from selected, blood bank eligible donors

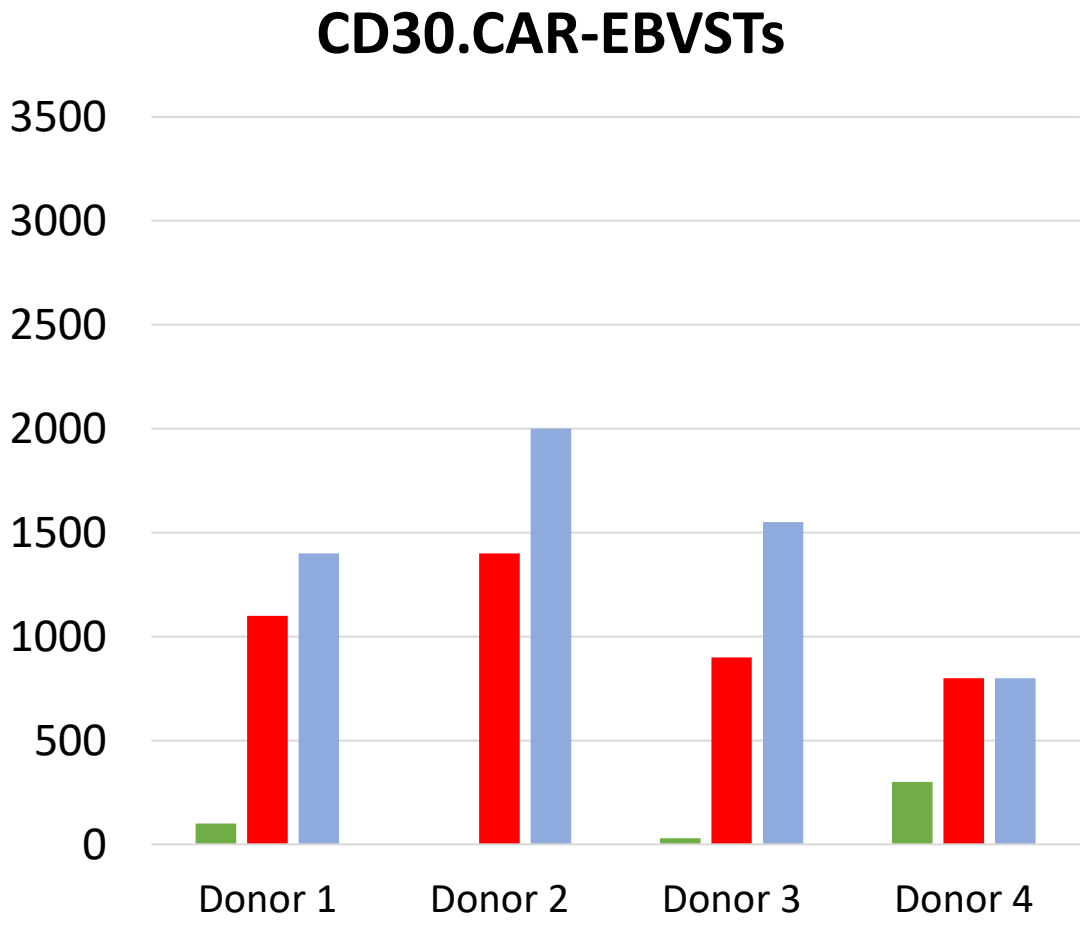
	Cell Numbers	Cell Viability >70%	Transduction >50%	Vector copy Number <5 per cell
Line 1	5.07×10^9	95.6%	98.3%	1.69
Line 2	5.31×10^9	97.25%	77.2%	1.85
Line 3	5.23×10^9	98.6%	99.7%	0.98
Line 4	3.48×10^9	86.9%	99.6%	2.13

- 5×10^9 cells sufficient for 12 infusions at highest dose level
- Expect ~500 fold expansion over 16 days

CD30.CAR-EBVSTs retain EBV antigen specificity

IFNγ spot forming cells per 100,000 EBVSTs



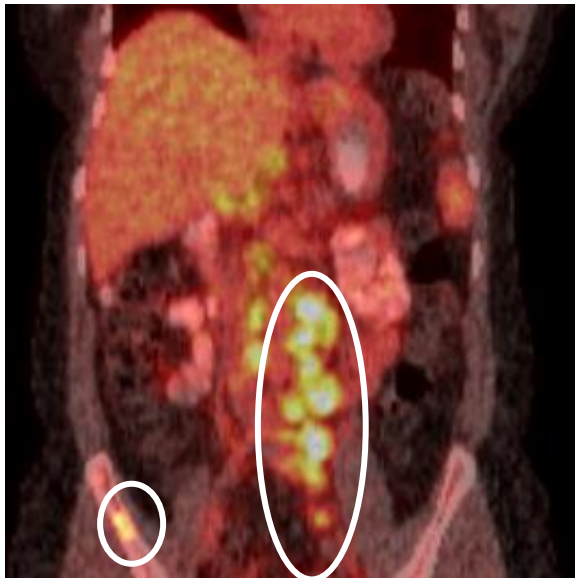


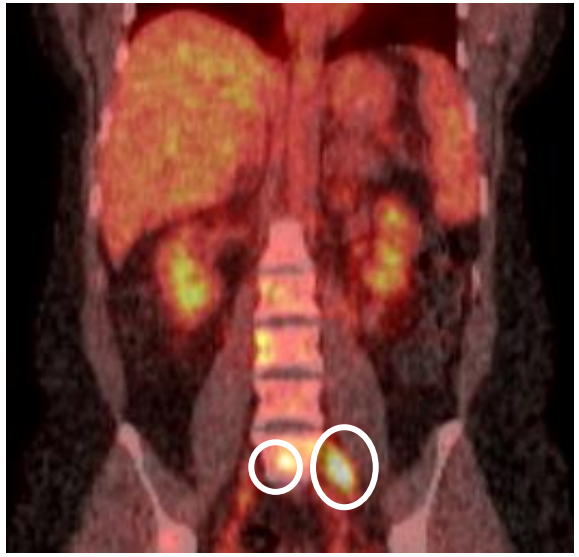
Patient characteristics - Hodgkin lymphoma

Patient	Age	Sex	# Prior Therapies	Prior Treatments
#1	34	F	5	ABVD, ICE, HDT/ASCT, brentuximab vedotin (BV), nivolumab
#2	47	M	5	ABVD, ESHAP, HDT/ASCT, BV, pembrolizumab
#3	29	M	6	ABVD, ICE, HDT/ASCT, BV, nivolumab, BV+bendamustine
#4	53	M	5	ABVD+COPP, BV, nivolumab, everolimus, bendamustine
#5	39	F	3	ABVD, nivolumab, BV+nivolumab
#6	37	M	4	ABVD+XRT, ICE, HDT/ASCT, BV
#7	29	F	5	ABVD, BV-ICE, HDT/ASCT, BV, bendamustine+gemcitabine+nivolumab
#8	44	F	6	ABVD, ICE, BV, BV+bendamustine, HDT/ASCT, pembrolizumab
#9 (#1)	35	F	7	ABVD, ICE, HDT/ASCT, BV, nivolumab, gemcitabine, BESTA
#10	24	F	4	ABVD, ICE, BV+nivolumab, everolimus+itacitinib
#11 (#6)	37	M	5	ABVD+XRT, ICE, HDT/ASCT, BV, BESTA

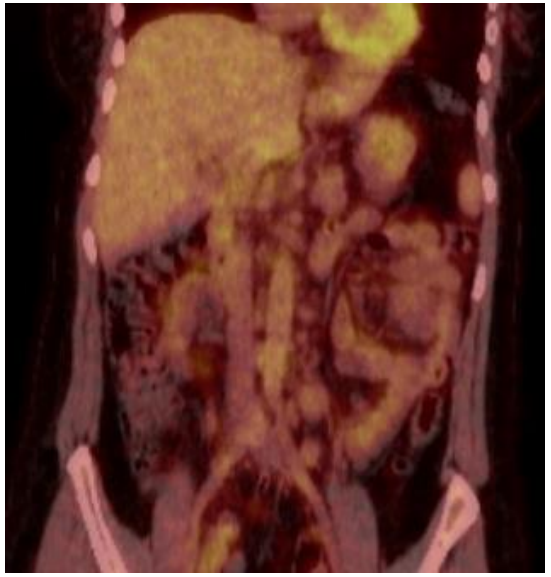
Patient #1, 34 y/o Female

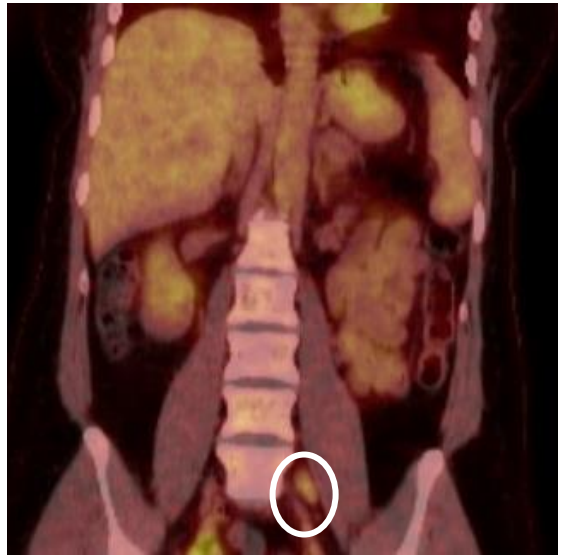
Pre-infusion
4 x 10^7
CD30.CAR-EBVSTs





Week 6

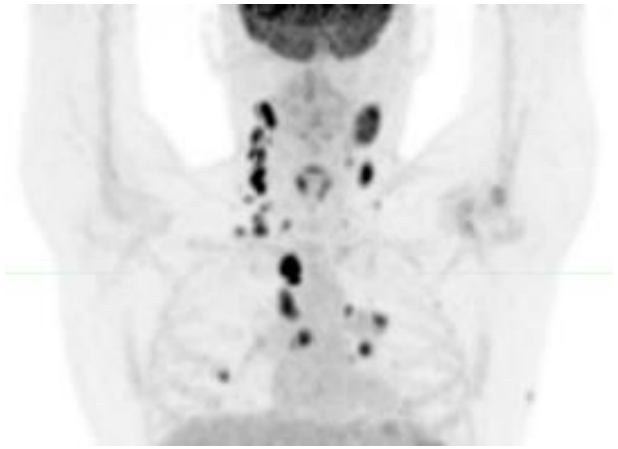


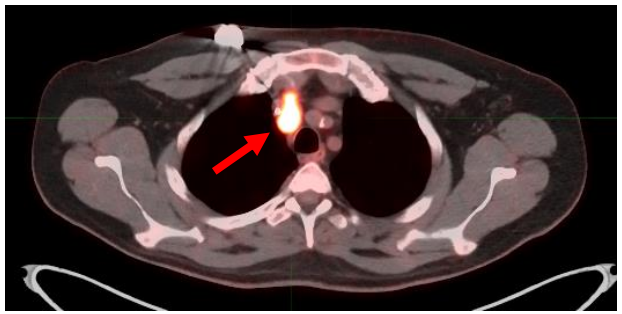


Resolution in most areas

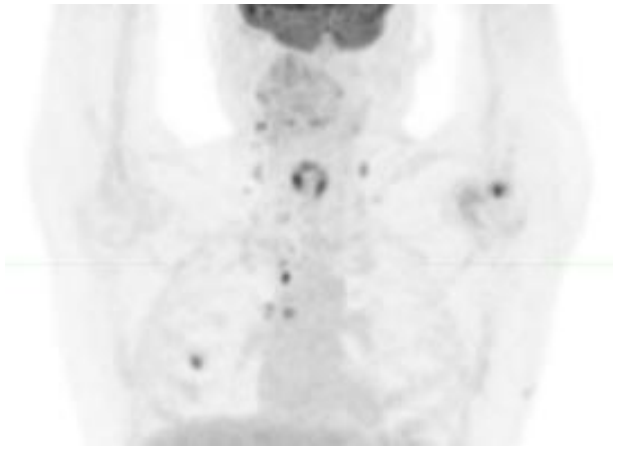
Patient #2, 47 y/o male

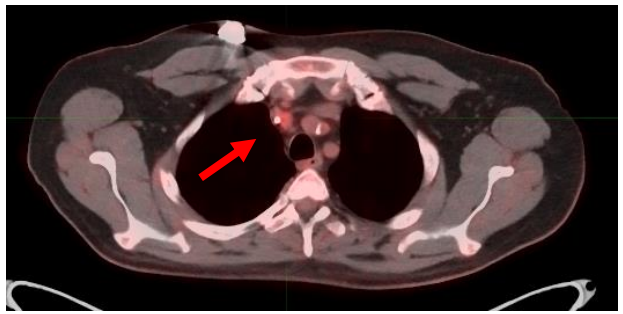
Pre-infusion





Week 6

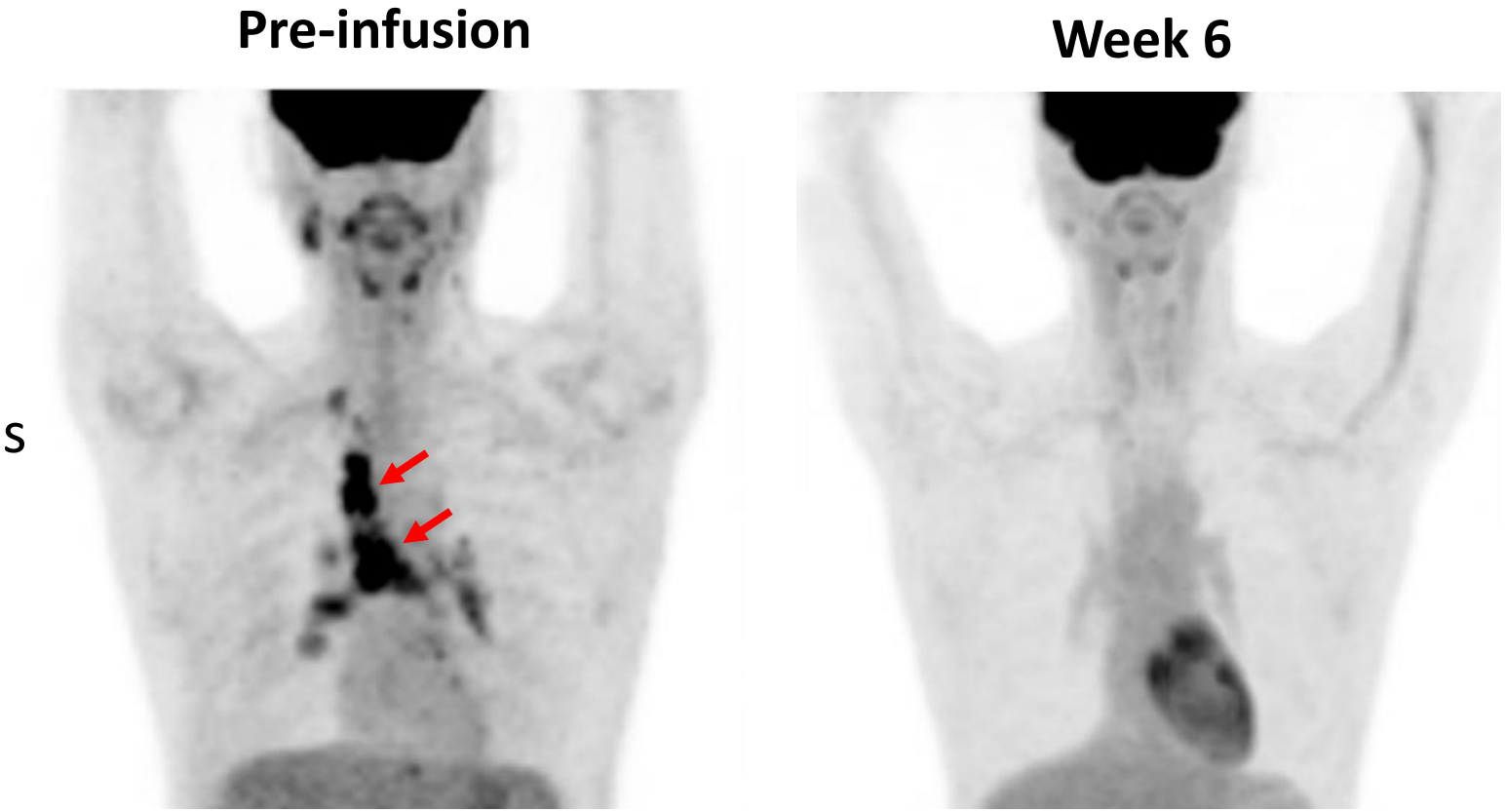




- Reduction of disease is seen in several areas
- Partial response

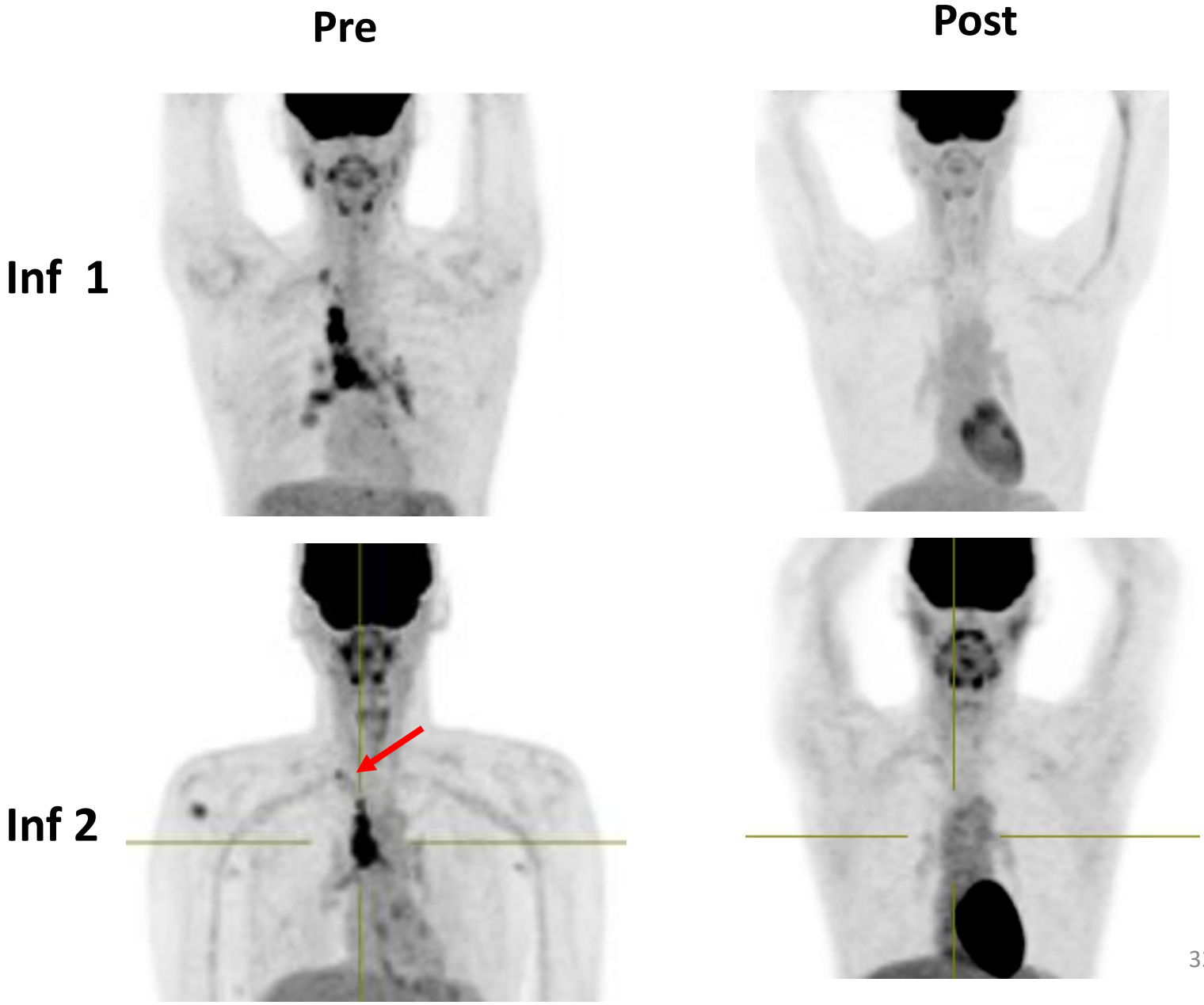
Clinical Response to CAR-EBVSTs (pt #6)

- 37y.o. male with relapsed Hodgkin lymphoma
- Dose level 2 , 1 x 10^8 T-cells
- Complete remission

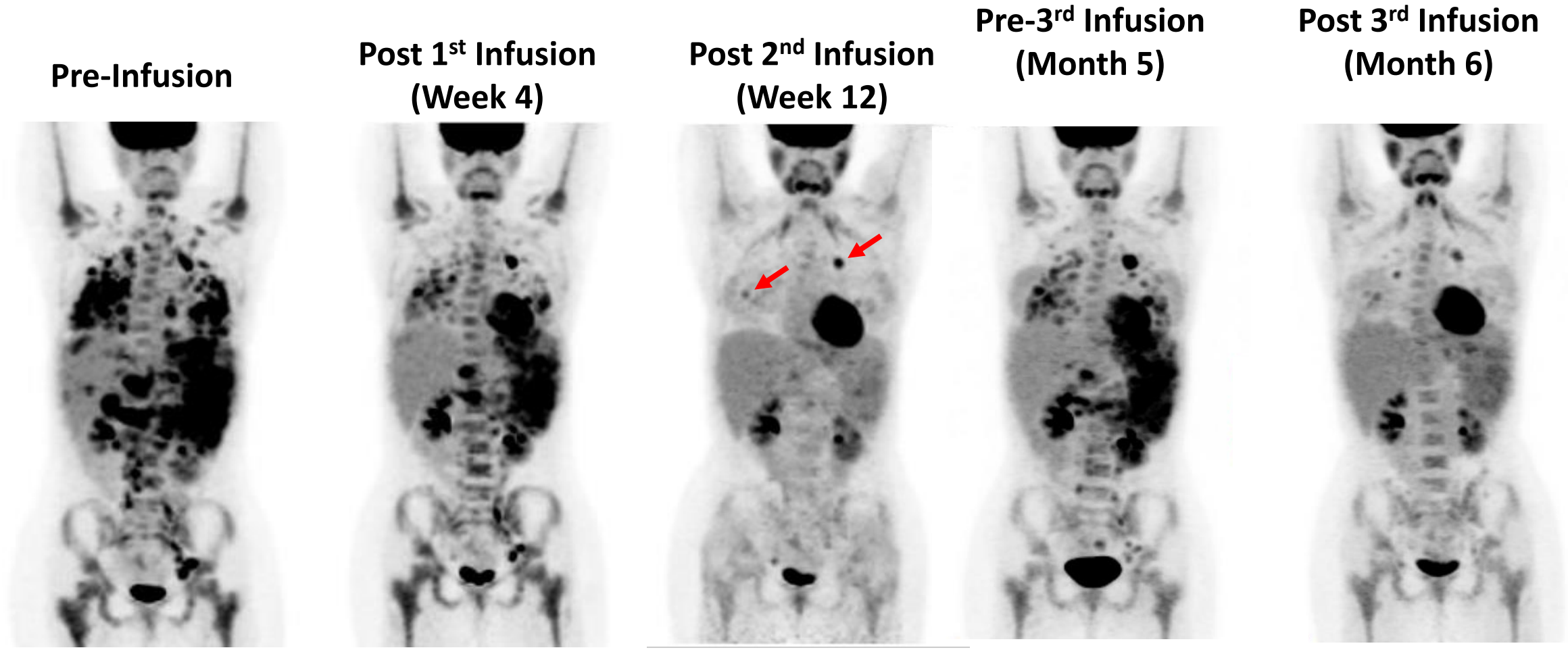


Repeat Infusions Are Effective (pt #6)

- Relapsed ~6 months after 1st infusion
- Re-enrolled on dose level 3 after 10 months
- 2nd complete response



Repeat Infusions Are Effective (pt #10)

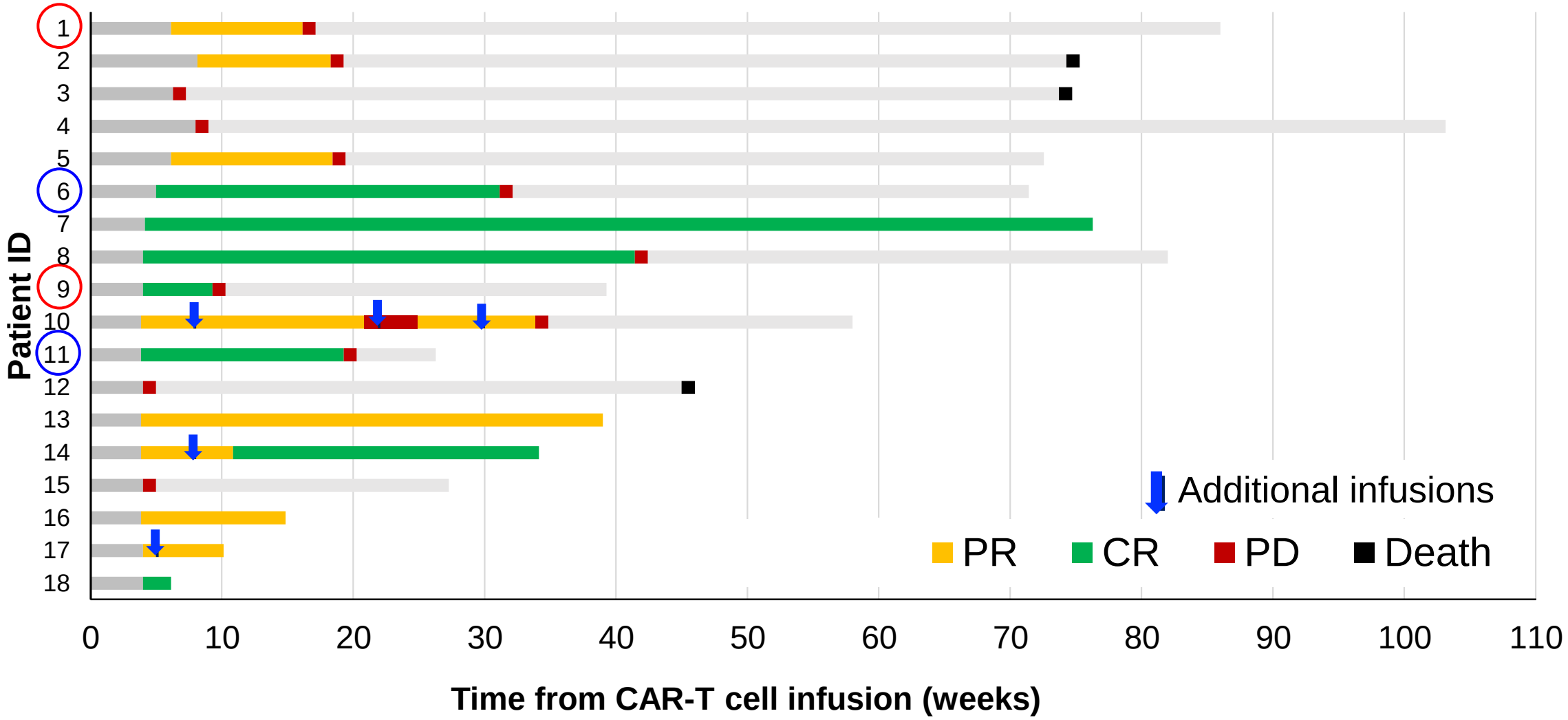


- 24y.o. female with Hodgkin lymphoma
- Dose level 3

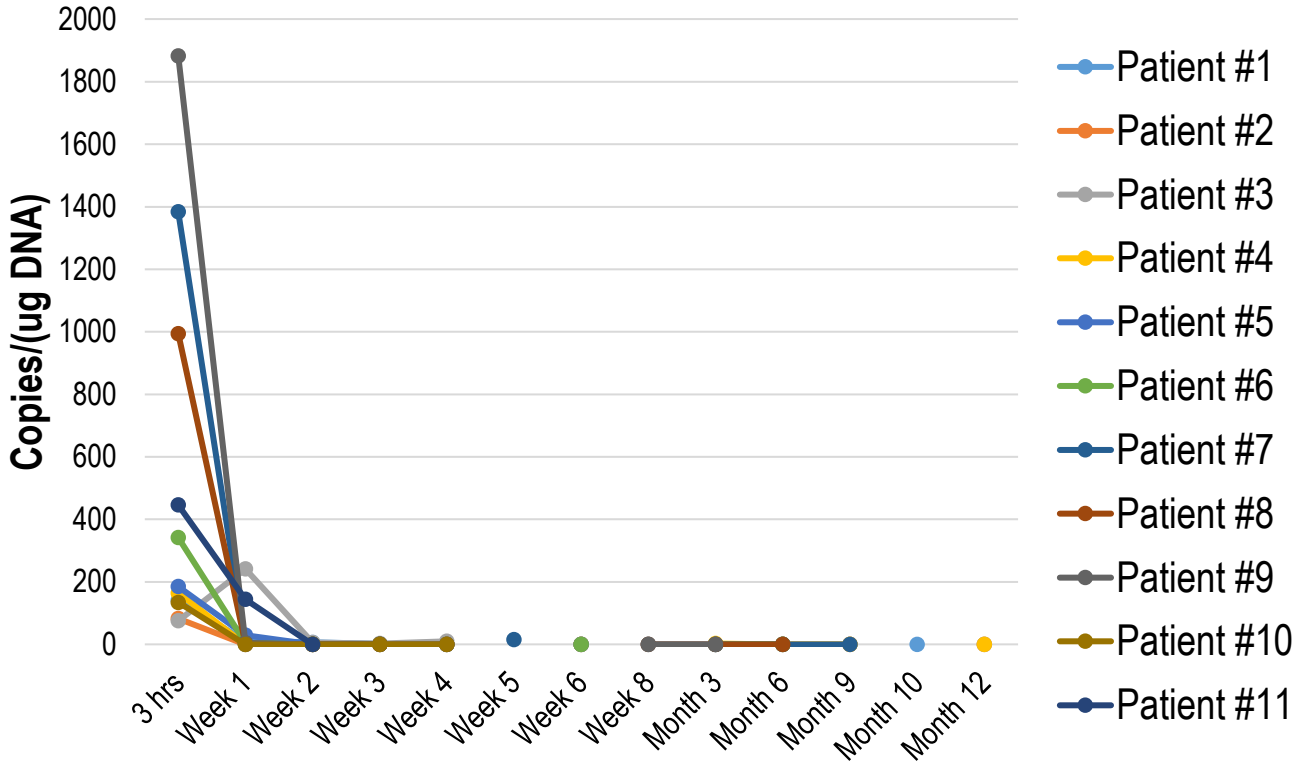
Patient Outcomes

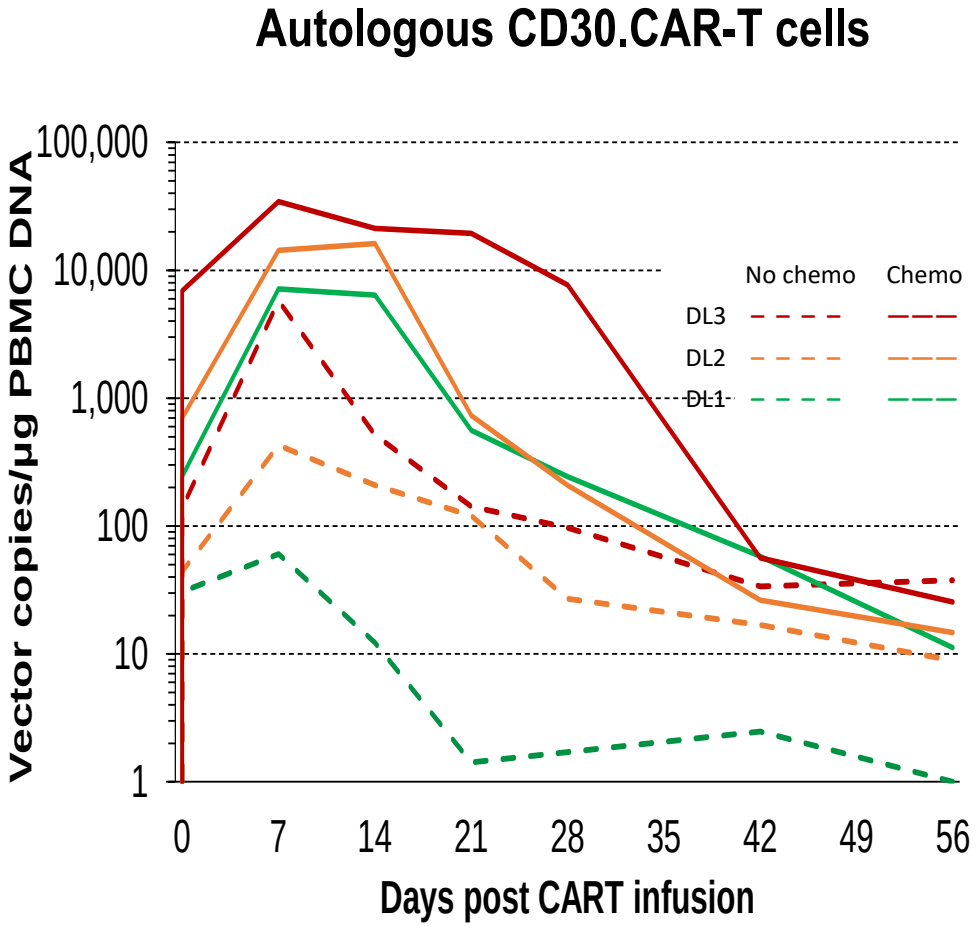
Patient	Dose Level	Line Infused	# Matches HLA (I,II)	CRS	Unexpected Severe Adverse Events (SAE)	Best Clinical Response
#1	1	#3	3,2	None	None	Partial response
#2	1	#3	2,0	None	None	Partial response
#3	1	#3	1,1	None	None	Progressive disease
#4	2	#5	1,1	None	None	Progressive disease
#5	2	#1	1,1	None	None	Partial response
#6	2	#2	1,0	None	None	Complete Response
#7	3	#1	2,2	None	Prolonged pancytopenia, Menorrhagia	Complete Response
#8	3	#3	2,1	None	None	Complete Response
#9 (#1)	3	#3	3,2	Grade 1	Prolonged pancytopenia	Complete Response
#10	3	#1	1,0	Grade 1	None	Partial response
#11 (#6)	3	#2	1,0	None	None	Complete Response
#12	3	#4	3,2	Grade 1	None	Progressive disease
#13	3	#3	2,0	Grade 1	None	Partial response
#14	3	#6, #2	2,2	None	None	Partial response, Complete Response

Timing and Duration of Responses



Limited Persistence of CD30.CAR-EBVSTs in Blood





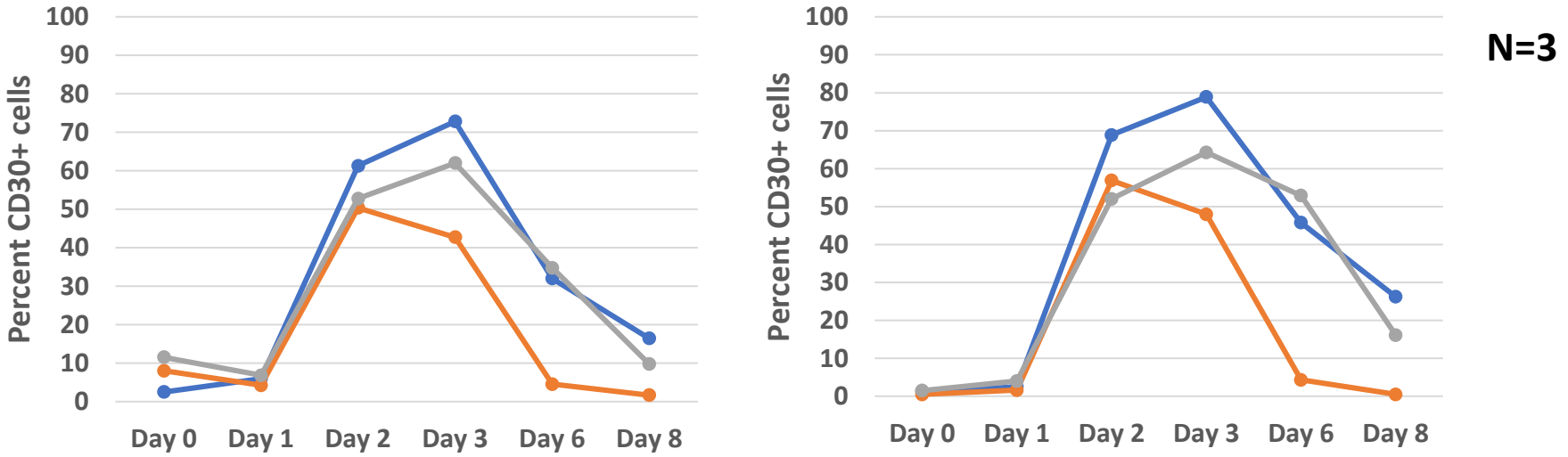
- CD30.CAR transgene detected with real time qPC..
- Rapid loss of CD30.CAR-EBVSTs in blood
- Autologous CD30.CAR-ATCs show expansion and persistence

Ramos CA, *et al.* JCI (2017) & JCO (2020)

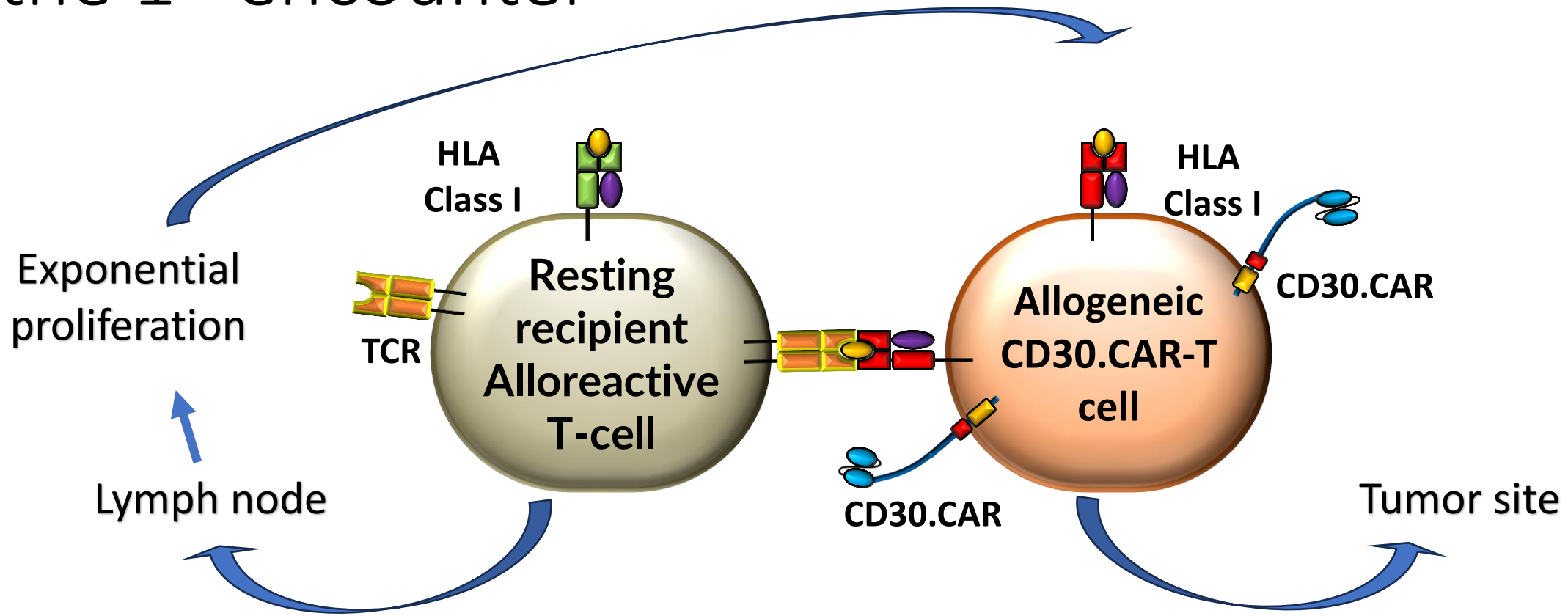
Reasons for lack of persistence

- Home to tumor site and remain?
- Allorejection
  - Number of alloreactive T-cells
    - ~4 x 10^{10}
  - Kinetics of CD30 upregulation (24 to 48 hours)

Kinetics of CD30 Expression after T-cell activation (CD3 and CD28 antibodies)

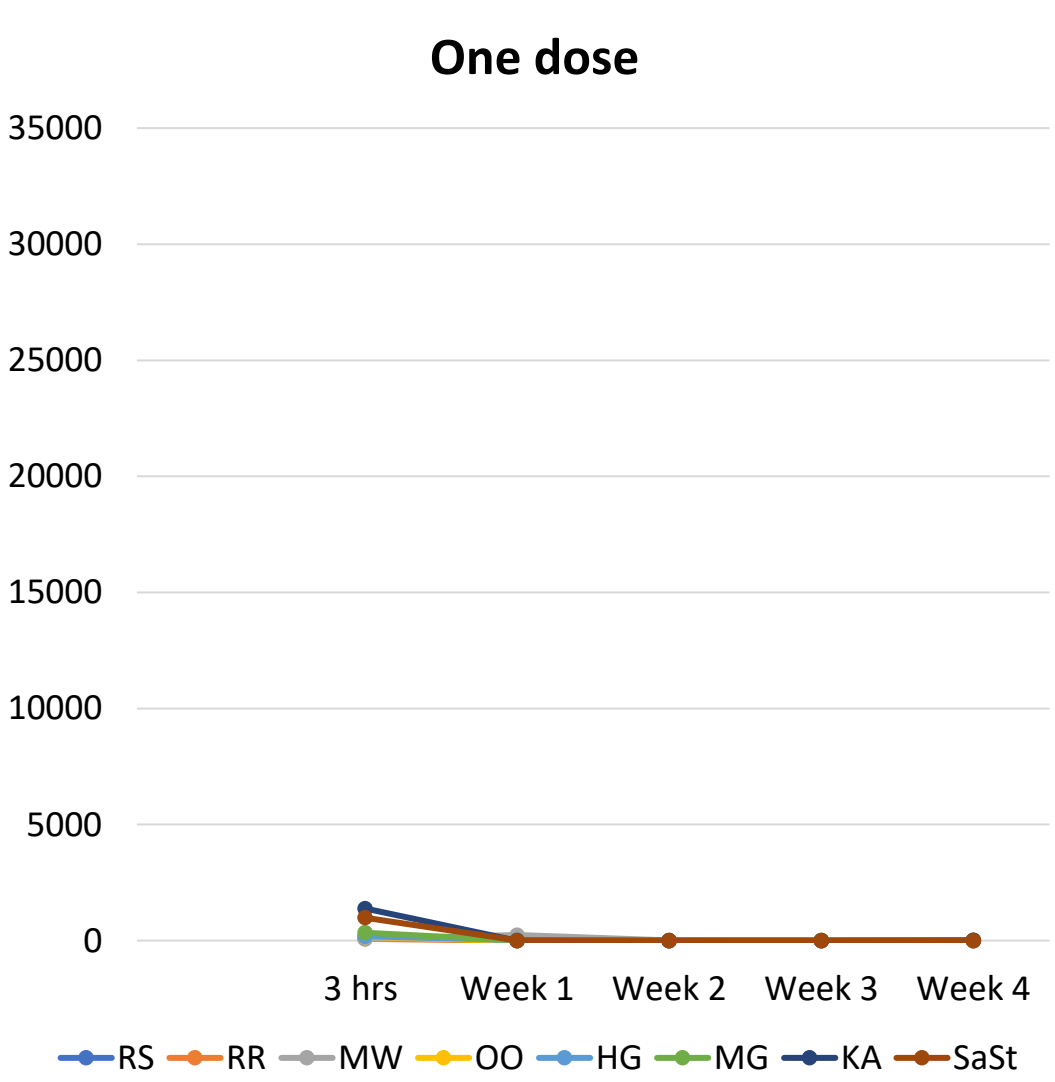


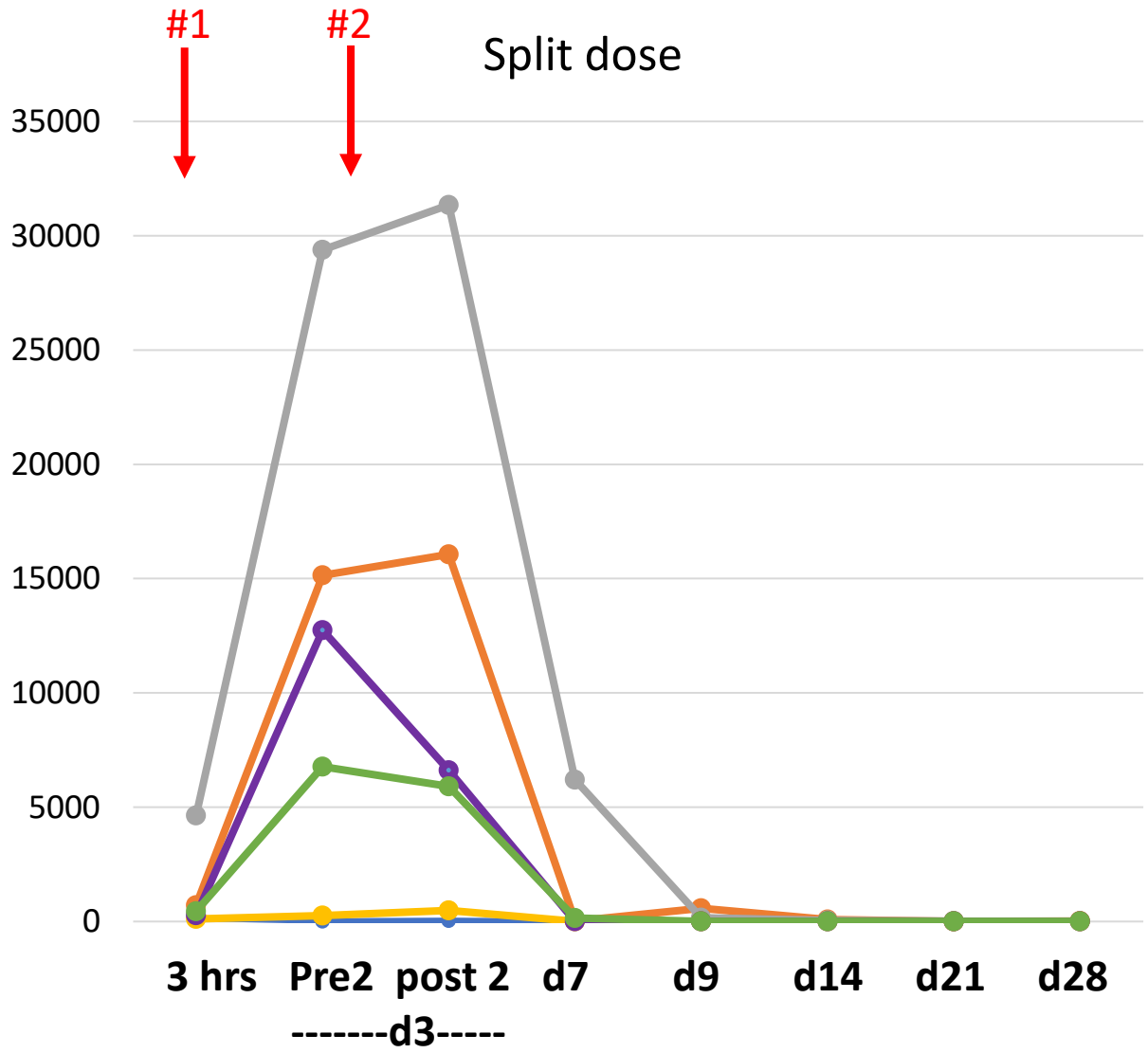
Alloreactive T-cell does not express CD30 on the 1st encounter



- **Evaluate split dosing on days 0 and 3**

CD30.CAR-EBVSTs do expand after infusion





How to improve response duration

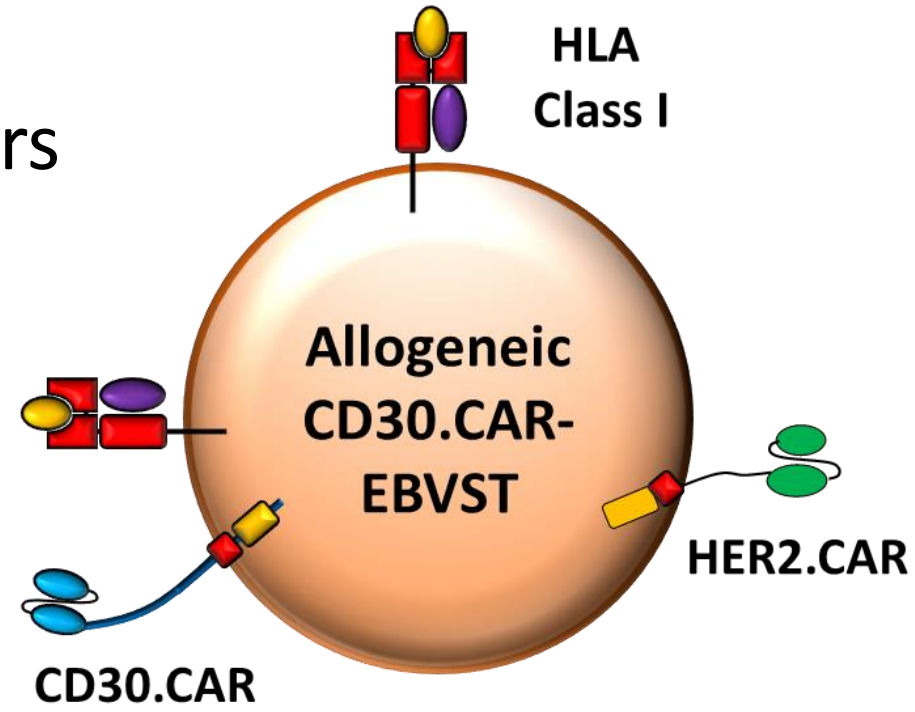
- Higher doses and repeat infusions
- Prolong cytokine support?
  - Lymphodepletion effects short lived
  - Constitutively active IL-7 receptor
    - IND in preparation
- Increase elimination of host alloreactive T-cells

How to eliminate armies of alloreactive T-cells?

- Antibodies to CD30
  - CD30.antibody toxin conjugate
    - Brentuximab vedotin (BV)
  - May kill potentially massive numbers of alloreactive T-cells
- How
  - Infuse allo CD30.CAR-EBVSTs to activate host alloreactive T-cells
  - 2 days later, infuse BV
  - One week later infuse second dose of CD30.CAR-EBVSTs
- Question
  - Does our CD30.CAR protect CD30.CAR-EBVSTs from BV

Extend application of off-the-shelf CD30.CAR-EBVSTs

- Other CD30+ malignancies
- Platform to treat other cancers
  - express additional CARs?



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